American Journal of Medical Genetics 69:380–382 (1997)

Analysis of 16 Cystic Fibrosis Mutations in Mexican Patients

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We carried out molecular analysis of 80 chromosomes from 40 unrelated Mexican patients with a diagnosis of cystic fibrosis. The study was performed in two PCR steps: a preliminary one to identify mutation ΔF508, the most frequent cause of cystic fibrosis worldwide, and the second a reverse dot-blot with allele-specific oligonucleotide probes to detect 15 additional common mutations in the Caucasian population. A frequency of 45% for ΔF508 was found, making it the most common in our sample of Mexican patients. Another five mutations (G542X, 3849 + 10 kb C→T, N1303K, S549N, and 621 + 1 G→T) were detected, and these accounted for 11.25%. The remaining mutations (43.75%) were undetectable with the methodology used. Am. J. Med. Genet. 69:380–382, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: cystic fibrosis; CFTR mutations; genetic diagnosis; Mexican CF patients

INTRODUCTION

Cystic fibrosis (CF) is the most common, early lethal genetic disease among Caucasians. Even though a cure is still not available, the life expectancy for CF patients has improved dramatically in the last 20 years [Welsh et al., 1995], due to both earlier diagnosis and more effective clinical intervention.

ΔF508 is the most common mutation causing CF, and is a deletion of a single phenylalanine codon from position 508 of the cystic fibrosis transmembrane regulator (CFTR) gene [Kerem et al., 1989]. This mutation accounts for 66% of CF chromosomes examined worldwide. Studies have found over 500 presumed mutations and close to 100 DNA polymorphisms in this gene. According to data from the Cystic Fibrosis Genetic Analysis Consortium [1994] (CFGAC), the most frequent non-ΔF508 mutations are the following: G542X (2.4%), G551D (1.6%), N1303K (1.3%), W1282X (1.2%), R553X (0.7%), 621 + 1 G→T (0.7%), 1717 – 1 G→T (0.6%), R117H (0.3%), R1162X (0.3%), G85E (0.2%), R347P (0.2%), ΔI507 (0.2%), and 3849 + 10 kb C→T (0.2%).

Other mutations are extremely rare, and the mutations in about 10% of defective chromosomes are still unknown. The frequencies of each vary among different populations, geographically and ethnically [CFGAC, 1994].

The identification of CF mutations by the polymerase chain reaction (PCR) has facilitated the study of population dynamics of ΔF508, the distribution of the other major mutations in different ethnic groups, and correlations between the genotype and clinical manifestations. The severe clinical manifestations of the disease, such as pancreatic insufficiency (PI) and meconium ileus, have been associated with ΔF508 [Johansen et al., 1991; Kerem et al., 1990]; likewise, a classification of severe or mild with respect to pancreatic insufficiency/sufficiency of a group of non-ΔF508 mutations has been developed [Kristidis et al., 1992].

Recently, molecular genetic studies were initiated in Latin American populations [Rojas et al., 1992; Orozco et al., 1993]. In Mexico there is a fusion of two ethnic groups which began 500 years ago with the arrival of the Spaniards. We investigated the genetic contribution of this well-characterized European group in our country to infer the influence of possible native alleles, which is poorly understood genetically. This analysis will constitute a useful reference for similar studies carried out on Mexican-Americans and for genetic counseling efforts underway in this latter ethnic group.

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Received 12 January 1996; Accepted 30 August 1996

MATERIALS AND METHODS

Samples of fresh peripheral blood (EDTA anticoagulated) were collected from 40 patients with a clinical diagnosis of CF, 34 of whom had at least one positive iontophoresis sweat test (>60 meq/l of chloride). Three patients had normal sweat tests; however, they suffered from a chronic respiratory disease such as recurrent pneumonia and atelectasis. For 3 patients in whom it was not possible to perform iontophoresis sweat testing, clinical symptoms were very suggestive of CF. In fact, the diagnosis in one was confirmed later by autopsy findings. Pancreatic insufficiency was diagnosed on a clinical basis by abnormal fecal fat (steatorrhea) and failure to gain weight and linear growth in 27 of the patients. All 40 patients were from unrelated Mexican families.

The samples were obtained from Guadalajara (Western), Monterrey (Northeast), and Puebla (Central). The referring physicians were asked to gather clinical data from the patients' families with their authorized consent.

Leukocytic DNA was obtained by standard methods [Herrmann and Frischauf, 1987]. An initial PCR was performed to detect the ΔF508 mutation, which was visualized by polyacrylamide gel electrophoresis [Rommens et al., 1990]. A second PCR assay was carried out with those samples that were negative for ΔF508 or that had a heterozygous genotype (ΔF508/unknown). The screening of 15 additional mutations was done using a multiplex PCR coupled to reverse dot-blot hybridization [Saiki et al., 1989; Chehab and Wall, 1992].

RESULTS AND DISCUSSION

Chromosomes (n = 80) in patients from Northeast (27 patients), Western (5 patients), and Central Mexico (8 patients) were studied. ΔF508 and five non-ΔF508 mutations were found in our genetic screening, accounting for 56.25% of CF chromosomes. The geographic distribution of the detected mutations is shown in Table I, and these results are compared to data from the CFGAC [1994].

Pancreatic function was determined in the patients on a clinical basis. A correlation between PI and the genotype was found. The 12 ΔF508 homozygotes identified in our study had clear clinical evidence of CF, with multisystemic involvement. Pancreatic insufficiency (PI) was found in all 12, while 5 of 10 ΔF508/unknown heterozygotes presented PI. All patients with two severe mutations suffered PI (ΔF508/G542X, G542X/S549N, and ΔF508/ΔF508). Two patients who had a compound genotype with one mild (3849 + 10 kb C→T) and one severe (ΔF508 and 621 + 1 G→T) mutation had PS. These data are in agreement with previous observations [Kristidis et al., 1992]. Of the two G542X/unknown heterozygotes, one had PI and the other had pancreatic sufficiency (PS). The N1303K/unknown heterozygote had a history of meconium ileus and PI. These results are shown in Table II.

The screening of CFTR gene mutations with a 16-mutation detection system allowed us to identify 56.25% of mutant alleles (45/80), while 43.75% remained undetected (35/80). In spite of the limited sample size, a general pattern of the distribution of CFTR gene mutations in different areas of the country could be seen. The frequency of the ΔF508 mutation found in this study (45%) is slightly lower than that reported in Spain (60%) [Chillón et al., 1990; Peral et al., 1991] and other European Mediterranean countries (55%) [Nunes et al., 1991], and similar to that found for Latin Americans living in the US (45%) [Grebe et al., 1994; CFGAC, 1994]. The frequency of the ΔF508 mutation in a previous report of Mexican CF patients of Central Mexico (39%) [Orozco et al., 1993] is similar to the value found in our study.

The G542X mutation is second in frequency after ΔF508, as has been reported worldwide and for Spain, where it has a frequency of 8.0% [Casals et al., 1993]; this is similar to the 4.8% value reported for Southern Europeans, specifically Italians [Nunes et al., 1991].

The third mutation found in our study was 3849 + 10 kb C→T, with a frequency of 2.5%; this was first reported in the Ashkenazi Jewish population with a frequency of 6.3% [Shoshani et al., 1992], and recently was found in the Hispanic population in the Southwestern US with a value of 2.3% [Grebe et al., 1994], which is very similar to our finding. This frequency is higher than the 0.2% reported by the CFGAC [1994]. The 2 patients whose genotype involves this mutation had PS; the sweat test was positive in one and normal in the other.

Mutations N1303K, S549N and 621 + 1 G→T had same frequency (1.25%) which was the lowest detected in our sample. N1303K mutation has been reported in

TABLE I. Mutation Frequency Data and Geographic Distribution of the Mutations Found in 80 Chromosomes From Mexican CF Patients

	Northeast n = 54		Central n = 16		Western n = 10		Total n = 80		
Mutation	n	(%)	n	(%)	n	(%)	n	(%)	CFGAC [1994] (%)
ΔF508	27	(50)	2	(12.5)	7	(70)	36	(45)	66
G542X	2	(3.7)	2	(12.5)		0	4	(5)	2.4
3849 + 10 kb C→T	1	(1.9)		0	1	(10)	2	(2.5)	0.2
N1303K		0	1	(6.25)		0	1	(1.25)	1.3
S549N		0	1	(6.25)		0	1	(1.25)	0.1
621 + 1 G→T		0		0	1	(10)	1	(1.25)	0.7
Other[a]	24	(44.4)	10	(62.5)	1	(10)	35	(43.7)	
Detected	30	(55.6)	6	(37.5)	9	(90)	45	(56.3)	

[a]Different from W1282X, R117H, R334W, R347P, A455E, ΔI507, 1717 – 1 G→T, G551D, R553X, and R560T.

TABLE II. Genotype vs. Phenotype Correlation in 40 Mexican CF Patients*

Genotype		Phenotype	
Allele 1	Allele 2	PI	PS
ΔF508	ΔF508	12	0
ΔF508	G542X	1	0
ΔF508	3849 + 10 kb C→T	0	1
ΔF508	Other[a]	5	5
G542X	Other[a]	1	1
G542X	S549N	1	0
N1303K	Other[a]	1	0
621 + 1 G→T	3849 + 10 kb C→T	0	1
Other[a]	Other[a]	6	5

*PI, pancreatic insufficiency; PS, pancreatic sufficiency.
[a]Different from W1282X, R117H, R334W, R347P, A455E, ΔI507, 1717 – 1 G→T, G551D, R553X, and R560T.

Southern Europeans as the fourth most common mutation with a frequency of 3.2% [Nunes et al., 1991].

It is interesting that we found one S549N mutant chromosome, which has a frequency of 0.1% worldwide. In a recent analysis carried out in the US with 764 CF chromosomes, this mutation was found only once, having a frequency of 0.1% [Shuber et al., 1993]. To confirm if this mutation is more frequent in our population, a larger screening study is needed.

We venture to say that the large number of unknown mutations found in this study (43.75%) would include Amerind alleles carrying as-yet undiscovered CF mutations.

PCR-based methodologies have notably improved the molecular diagnosis of CF and now make possible the detection of carriers. They have very high specificity and are quickly being incorporated as a clinical tool. All these advantages have also benefited carrier detection programs, prenatal diagnosis, and even preimplantation diagnosis for an effective prevention of the disease [Handyside and Winston, 1992].

ACKNOWLEDGMENTS

We thank Prof. R.M. Chandler-Burns for his critical reading of our manuscript, Raquel Cardiel for manuscript typing, and Alma Rosa Cruz and Dolores Esquivel for technical assistance. We also thank the Global Network for Molecular and Cell Biology of UNESCO for support.

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